

22. (Amended) A method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a single cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, the method comprising the steps of:

- MG1
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- a. coating paramagnetic particles or beads with [an] a monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell and not on a non-target-cell in the cell mixture;
 - b. mixing the coated paramagnetic particles or beads with the cell suspension containing the target-cells;
 - c. incubating the mixture under gentle rotation at about 4°C until target cell-bead rosettes are formed; and
 - d. quantitating the target cell-bead rosettes after incubation[examining the target-cells after incubation; and
 - e. counting the target-cells after incubation].

23. (Amended) The method of claim 22, wherein the paramagnetic particle or bead is coated with a monoclonal murine or a human antibody or fragment thereof.

28. (Amended) The method of claim 22, wherein [when the target cell population is contained in blood or bone marrow aspirates,] the method further comprises the step of:
pre-incubating the antibody-coated paramagnetic particle and the cell suspension with mild detergent.

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29. (Amended) The method of claim 28, wherein the preincubating comprises as detergent [Tween 20™] polyoxyethylenesorbitan monolaurate at a concentration less than 0.1% and the preincubation lasts 30 minutes at 4°C.

E3
33. (Amended) The method of claim 22, further comprising the steps of:
isolating the [target-cells by exposing the complex of cells and paramagnetic
particles to] target cell-bead rosettes by applying a magnetic field to separate the rosettes
[magnetically aggregate the cells;
subjecting the magnetically aggregated cells to further biological, biochemical,
and immunological examination].

Sub 2
34. (Amended) The method of claim 22, wherein the ^{UM3} monoclonal antibody or
fragment thereof is directed against an antigen ~~or a receptor~~ in a cell with abnormal
developmental patterns.

36. (Amended) The method of claim 22, wherein the monoclonal antibody or
fragment is of IgG isotype, a F(ab')₂ fragment, a F(ab) fragment, IgM, or a fragment of IgM.

E4
37. (Amended) The method of claim 22, wherein the mixed cell [suspension or]
population comprises mammalian tissue, a pleural effusion, a peritoneal effusion, a body fluid, or
a solid tumor in a normal tissue or organ.

39. (Amended) The method of claim 22, wherein the monoclonal antibody or
antibody fragment is directed against fibronectin receptor, β -integrin, vitronectin receptor, $\alpha\gamma\beta 3$ -
integrin, P-selectin, GMP-140, CD44-variants, N-CAM, E-cadherin, Le^x, CEA, EGF receptor, c-
erbB-2, HER2, transferin receptor, TNF-receptor, high molecular weight antigen (HMW
250,000), p95-100, TP-1 and TP-3 epitope, [Mv 200kD, Mv160kD, MOC-31 epitope, cluster 2
epithelial antigen, MUC-1 antigen, DF3-epitope, gp290kD, prostate high molecular antigen
(Mv>400kD), TAG 72, bladder carcinoma antigen (Cancer Res. 49, 6720, 1989), Mv 48kD
colorectal carcinoma antigen, lung carcinoma antigen Mv 350-420kD, Mel-14 epitope, β_2 -
microglobulin, Apo-1 epitope, or pan-human cell antigen.

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40. (Amended) The method of claim 22, wherein the monoclonal antibody or antibody fragment is directed against a growth factor receptor [and] or an oncogene product expressed on the membrane of a malignant cell.

41. (Amended) The method of claim 40, wherein the monoclonal antibody or antibody fragment is directed against an insulin receptor, an insulin-like receptor, or FGF. *? second*

ES
42. (Amended) The method of claim 34, wherein the monoclonal antibody or antibody fragment is directed against an adhesion membrane molecule or an MDR protein[s] in the abnormal cell. *? second ?*

Sub 43
43. (Amended) The method of claim 34, wherein the monoclonal antibody or antibody fragment is directed against breast, ovarian or lung carcinoma cells; melanoma, sarcoma, glioblastoma or cancer cells of the gastrointestinal tract; melanoma, sarcoma, glioblastoma or cancer cells of the genitourinary tract; or melanoma, sarcoma, glioblastoma or cancer cells of the reticuloendothelial system.

46. (Amended) A kit for performing the method of claim 22, the kit comprising:
a. a specific monoclonal antibody or antibody fragment directed to an antigen on a target-cell, which monoclonal antibody or fragment is ^{coated on} (effective for coating) a paramagnetic particle or bead without removing its antigen-binding ability;
b. a paramagnetic particle or bead; and
c. [another] a second specific monoclonal antibody or antibody fragment directed against an antigen or a receptor within or on the target cell;
wherein said [another] second antibody or antibody fragment is conjugated to [biotin or to an enzyme; or wherein said another antibody or antibody fragment is bound to a non-paramagnetic particle with a specific color or with a bound enzyme] a detectable label.

Fig 4
47. (Amended) The kit of claim 46, wherein the detectable label is an enzyme [is] peroxidase or alkaline phosphatase.

48. (Amended) A method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a single cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, the method comprising the steps of:

a. [pre-]coating paramagnetic particles or beads with [an] a first antibody directed against an Fc-portion of [an] a second monoclonal antibody or antibody fragment [directed against a membrane structure specifically expressed on the target-cell and not on a non-target-cell in the cell mixture];

b. forming a complex comprising the pre-coated paramagnetic particles, the antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell and not on a non-target-cell in the cell mixture, and the target-cell;

c. examining the target-cells in the complex; and

d. counting the target-cells in the complex]

b. mixing the coated paramagnetic particles with the second monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target cell and the cell suspension.

c. incubating the mixture under gentle rotation at about 4°C until target cell-bead rosettes are formed; and

d. quantitating the target cell-bead rosettes after incubation.

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51.

(Amended) The method of claim [49] 48, wherein incubating lasts 30 minutes.

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(Amended) The method of claim 48, wherein the second monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell [and not on a non-target-cell in the cell mixture] is a murine or a human antibody or fragment thereof.

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(Amended) The method of claim 48, wherein [when the target cell population is contained in blood or bone marrow aspirates,] the method further comprises the step of:

pre-incubating the first antibody-coated paramagnetic particle and the cell suspension with mild detergent.

61. (Amended) The method of claim [48] 60, wherein the preincubating comprises as detergent [Tween 20™] polyoxyethylenesorbitan monolaurate at a concentration less than 0.1% and the preincubation lasts 30 minutes at 4°C.

62. (Amended) The method of claim 48, wherein when the density of target-cells is low, or when the ratio of target cell/total cells in the cell mixture is low ($\leq 1\%$), the method further comprises [the step of subjecting the complex to] after incubating, applying a magnetic field to separate out the target/cell-bead rosettes.

64. (Amended) The method of claim 48, wherein [the step of examining, the step of counting, or both steps comprise] quantitating includes counting the target bead rosettes using a microscope or a cell or particle counting device.

66. (Amended) The method of claim 48, wherein the second monoclonal antibody or fragment thereof is directed against an antigen or a receptor[s] in a cell[s] with abnormal developmental patterns.

71. (Amended) The method of claim 48, wherein the antibody or antibody fragment is directed against fibronectin receptor, β -integrin, vitronectin receptor, $\alpha\gamma\beta 3$ -integrin, P-selectin, GMP-140, CD44-variants, N-CAM, E-cadherin, Le^x, CEA, EGF receptor, c-erbB-2, HER2, transferin receptor, TNF-receptor, high molecular weight antigen (HMW 250,000), p95-100, TP-1 and TP-3 epitope[,] Mv 200kD, Mv160kD, MOC-31 epitope, cluster 2 epithelial antigen, MUC-1 antigen, DF3-epitope, gp290kD, prostate high molecular antigen (Mv>400kD), TAG 72, bladder carcinoma antigen (Cancer Res. 49, 6720, 1989), Mv 48kD colorectal carcinoma antigen, lung carcinoma antigen Mv 350-420kD, Mel-14 epitope, β_2 -microglobulin, Apo-1 epitope, or pan-human cell antigen.

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72. (Amended) The method of claim 48, wherein the second monoclonal antibody or antibody fragment is directed against a growth factor receptor [and] or an oncogene product expressed on the membrane of a malignant cell.

74. (Amended) The method of claim 66, wherein the second monoclonal antibody or antibody fragment is directed against an adhesion membrane molecule or an MDR protein[s] in the abnormal cell.

E 12
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75. (Amended) The method of claim 66, wherein the second monoclonal antibody or antibody fragment is directed against breast, ovarian or lung carcinoma cells; melanoma, sarcoma, glioblastoma or cancer cells of the gastrointestinal tract; melanoma, sarcoma, glioblastoma or cancer cells of the genitourinary tract; or melanoma, sarcoma, glioblastoma or cancer cells of the reticuloendothelial system.

78. (Amended) A kit for performing the method of claim 48, the kit comprising:
a. a first [specific] monoclonal antibody or antibody fragment directed [to] against a membrane structure specifically expressed on the target-cell [and not on a non-target-cell in the cell mixture];
b. a second antibody [or antibody fragment] directed [to] against an Fc-portion of the first monoclonal antibody or fragment thereof[, which second antibody or fragment is effective for coating a paramagnetic particle or bead without removing its antigen-binding ability];

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MA 65
c. a paramagnetic particle or bead; and
d. a labeled third specific monoclonal antibody [or antibody fragment] directed against an antigen or a receptor within or on the target cell[;

wherein said third antibody or antibody fragment is conjugated to biotin or to an enzyme; or wherein said third antibody or antibody fragment is bound to a non-paramagnetic particle with a specific color or with a bound enzyme].

813 ~~79.~~ (Amended) The ~~kit~~ of claim 78, wherein the label on the third monoclonal antibody is an enzyme [is] peroxidase or alkaline phosphatase.

Please add and consider new claims 80-107:

80. (New) A method according to claim 22 further comprising after incubating; detecting a second antigen of the target cell by adding a second labeled monoclonal antibody ~~directed to the second antigen~~ ^{NAB} to the cell suspension; and quantitating the amount of labeled second monoclonal antibody bound to the rosettes. ??

81. (New) The method according to claim 80, wherein the second monoclonal ^{second?} antibody is specific for a tumor prognostic marker. ?

82. (New) The method according to claim 80, wherein the second monoclonal antibody is labeled with fluoresceine, a radioactive compound, biotin, or an enzyme. ?

814 83. (New) A method according to claim 22, further comprising before mixing; prelabeling the target cells with a labeled second monoclonal antibody to second antigen on the target cell; and after incubating, quantitating the amount labeled second monoclonal antibody bound to the rosettes. ??

84. (New) A method according to claim 22, further comprising after incubating, applying a magnetic field to separate out the target cell bead rosettes; and detecting target cells specific genes at the DNA, mRNA or protein level.

85. (New) The method according to claim 84 wherein the detecting target cells specific genes is by using polymerase chain reaction.

86. (New) The method according to claim 84, wherein detecting target cell specific genes is by hybridization to a target cell gene specific probe.

87. (New) A method for detecting tumor cells in a cell suspension of mixed cell population or in a single cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, comprising:

- a) coating paramagnetic particles with a tumor-specific monoclonal antibody or fragment thereof;
- b) mixing the coated paramagnetic particles with the cell suspension;
- c) incubating the mixture at about 4°C under gentle rotation until tumor cell-bead rosettes are formed; and
- d) quantitating the number of tumor cell-bead rosettes.

88. (New) ^{the} A method according to claim 87 further comprising after incubating; applying a magnetic field to the mixture to separate out the tumor cell-bead rosettes.

89. (New) ^{the} A method according to claim 87, wherein the tumor-specific monoclonal antibody is specific for tumor antigens comprising a growth factor receptor, an oncogene product expressed on the membrane of a malignant cell, an adhesion membrane molecule, an MDR protein, breast, ovarian or lung carcinoma cells; melanoma, sarcoma, glioblastoma or cancer cells of the gastrointestinal tract; melanoma, sarcoma, glioblastoma or cancer cells of the genitourinary tract; or melanoma, sarcoma, glioblastoma or cancer cells of the reticuloendothelial system.

90. (New) The method of claim 87, wherein the monoclonal antibody or antibody fragment is directed against an insulin receptor, an insulin-like receptor, or FGF.

91. (New) A method according to claim 87 further comprising, after incubating; detecting a second antigen on the tumor cell by adding a labeled second monoclonal antibody specific for the second antigen to the cell suspension; and quantitating the amount of labeled second monoclonal antibody bound to the tumor cell-bead rosettes.

92. (New) A method of detecting metastatic cancer cells in a suspension of a mixed cell population or in a single cell suspension from a solid tissue when the metastatic cancer cells are present at less than 1% of the cell suspension, the method comprising the steps of:

- a) coating paramagnetic particles or beads with a cancer specific monoclonal antibody or antibody fragment;
- b) mixing the coated paramagnetic particles or beads with the cell suspension;
- c) incubating the mixture under gentle rotation at about 4°C until tumor cell-bead rosettes are formed;
- d) applying a magnetic field to separate out the tumor cell-bead rosettes; and
- e) quantitating the tumor cell-bead rosettes after separation.

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E 14
93. (New) A method according to claim 87, wherein the tumor-specific monoclonal antibody is specific for tumor antigens comprising a growth factor receptor, an oncogene product expressed on the membrane of a malignant cell, an adhesion membrane molecule, an MDR protein, breast, ovarian or lung carcinoma cells; melanoma, sarcoma, glioblastoma or cancer cells of the gastrointestinal tract; melanoma, sarcoma, glioblastoma or cancer cells of the genitourinary tract; or melanoma, sarcoma, glioblastoma or cancer cells of the reticuloendothelial system.

94. (New) The method of claim 87, wherein the monoclonal antibody or antibody fragment is directed against an insulin receptor, an insulin-like receptor, or FGF.

95. (New) The method of claim 91, wherein incubating lasts for 5-10 minutes to 2 hours.

96. (New) A method according to claim 93, wherein the mixture is incubated for about 30 minutes.

97. (New) A method according to claim 91, wherein the tumor cell-bead rosettes are quantitated by counting them using a microscope or a cell or particle counting device.

98. (New) A method according to claim 91 further comprising after quantitating; culturing the tumor cell-bead rosettes in a growth medium until a cell culture is established.

99. (New) ~~The method of claim 48 further comprising after incubating; detecting a second antigen on a target cell by adding a labeled third monoclonal antibody specific for the second antigen on the target cell to the mixture; and quantitating the amount of labeled third monoclonal antibody bound to the target cell-bead rosettes.~~

Separate epitopes

100. (New) ~~A method according to claim 97,~~ wherein the labeled third monoclonal antibody is labeled with flouresceine, a radioactive compound, biotin or an enzyme. *NAB*

E14 101. (New) ~~A method according to claim 48, further comprising after incubating; applying a magnetic field to the mixture to separate out the target cell-bead rosettes; and detecting target cell specific genes.~~

102. (New) A method according to claim 99, wherein the target cell specific genes are detected using polymerase chain reaction.

103. (New) A method according to claim 99, wherein the target cell specific genes are detected using a target cell specific gene probe.

104. (New) A method according to claim 48 further comprising, after incubating; applying a magnetic field to the mixture to separate out target cell-bead rosette; and culturing the target cell-bead rosettes in a growth medium to establish a cell culture.

Sub 18 105. (New) ~~The method of claim 33, wherein quantitating includes counting the target bead rosettes using a microscope or a cell or particle counting device.~~